Social and sexual representation in the primary somatosensory cortex

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Abstract

Rats are nocturnal animals and henceforth rely heavily upon their sense of touch. This is primarily achieved by the use of large facial whiskers (macrovibrissae). The processing of touch in the primary somatosensory cortex of the vibrissae (barrel cortex) has been extensively studied using simple object stimuli. Recent evidence suggested that actively touching another rat elicits fundamentally different neuronal correlates as compared to actively touching a non-living object. Based on those findings, this thesis explores the neurophysiology of two forms of social relevant touch: facial touch and genital touch. In the first part, I investigated, how subthreshold activity is altered during whisking in a social context using *in vivo* whole-cell recordings in the barrel cortex of head-restrained rats. Whisking was associated with strong membrane potential (V_m) fluctuations during facial touch, but not during free whisking. In addition we found several non-tactile responses: (i) Strong whisking related V_m fluctuations could be seen even prior to contact and differed from those observed in free whisking episodes. Remarkably, such a pre-depolarization prior to touch was not observed in anaesthetized animals. (ii) The V_m fluctuations locked to the rat's whisking observed in interactions with awake conspecifics were larger than those seen for whisking onto different objects and a stuffed rat. (iii) The responses I observed were not correlated to whisking parameters. In summary, social facial touch induces responses in the barrel cortex that are remarkably different from responses evoked with conventional tactile stimuli.

The second part of the thesis characterized the anatomy and physiology of the rat genital cortex by combining receptive field mapping with cytochrome oxidase staining of cortical layer 4. Mapping experiments revealed a robust representation of the genitals in rat primary somatosensory cortex. Genital responses were more frequent in males than in females. Genital cortex neurons showed unusual, discontinuous and sexually dimorphic receptive fields. In males, genital neurons were mostly co-activated by tactile stimulation of the forearm, whereas female genital neurons were co-activated by tactile stimulation of the trunk area. Hence, body parts co-represented with genitalia are those parts contacted in males and females during mounting. In contrast to the physiological sexual dimorphism, cytochrome oxidase staining of layer 4 revealed a stunning monomorphism of the cortical penis and clitoris representation. This is a surprising finding given the pronounced dimorphism of external genitals. We also found that the relative size of genital cortex massively increases during puberty. Our findings provide insight into the hitherto little studied representation of sexual information in the cortex.

Zusammenfassung

Ratten sind nachtaktive Tiere und daher besonders auf ihren Tastsinn angewiesen. Hierbei werden Berührungen vorallem durch den Einsatz der großen Schnurrhaare (Makrovibrissen) wahrgenommen. Die Verarbeitung von Berührung in der primären somatosensorischen Hirnrinde der Vibrissen (Barrel Kortex) wurde ausgiebig durch taktile Stimulation mit einfach Objekten erforscht. Neuere Ergebnisse haben jedoch gezeigt, dass das Berühren einer anderen Ratte, fundamental verschiedene neuronale Antworten auslöst, als das Berühren eines Objektes. Darauf aufbauend untersucht diese Doktorarbeit die Neurophysiologie von zwei verschiedenen Formen von sozial relevanten Berührungen: Die Berührung von Artgenossen mit den Vibrissen und die Berührung der Genitalien. Im ersten Teil, habe ich durch *in vivo* Ganzzellableitungen vom Barrel Cortex in kopf-fixierten Ratten untersucht, wie die Membranpotentialaktivität durch das Berühren einer anderen Ratte beeinflusst wird. Während der Berührung durch Artgenossen waren die Vibrissenbewegungen mit starken Membranpotentialänderungen assoziiert. Bei der spontanen Vibrissenbewegung ohne taktilen Stimulus wurden diese Korrelationen nicht beobachtet. Die Zellantworten während sozialen Berührungen wurden nicht allein durch die taktile Stimulation per se ausgelöst: (i) Die mit der Vibrissenbewegung korrelierten Membranpotenzialfluktuationen traten bereits auf, bevor die Tiere sich berührten und unterschieden sich signifikant von solchen, die während spontanten Vibrissenbewegungen auftraten. Erstaunlicherweise, konnten die Membranpotenzialantworten vor der eigentlichen Berührung nicht in anesthetisierten Ratten beobachtet werden. (ii) Die mit der Vibrissenberührung korrelierten Membranpotenzialfluktuationen waren von der Amplitude her größer, wenn die kopf-fixierten Tiere mit einem Artgenossen interagierten, verglichen zu Interaktionen mit Nichtartgenossen. (iii) Die beobachteten Antworten zeigten keine Korrelation mit dem Berührungsverhalten während sozialer Interaktionen. Daher lässt sich zusammenfassen, dass eine Berührung der Vibrissen durch einen Artgenossen, neuronale Antworten im Barrel Kortex auslöst, die sich erheblich von Antworten unterscheiden, die durch konventionelle taktile Stimuli ausgelöst wurden.

Der zweite Teil der Doktorarbeit untersucht den Genital Kortex der Ratte. Diese sensorische Region wurde in der Arbeit erstmals physiologisch und durch Cytochromoxidase Färbunug von Schicht 4 identifiziert. Die elektrophysiologische Charakterisierung der rezeptiven Felder demonstrierte eine robuste Repräsentation der Genitalien im somatosensorischem Kortex. Neuronale Antworten waren häufiger im Genital Kortex von Männchen zu finden, als in dem von Weibchen. Neurone im Genital

Kortex zeigten ungewöhnliche, diskontinuierliche und sexuell dimorphe rezeptive Felder. In Männchen, zeigten Neurone hauptsächlich eine Co-Aktivierung durch die taktile Stimulation des Vorderarms, während die Nervenzellen im Genital Kortex von Weibchen eher eine Co-Aktivierung durch die taktile Stimulation des Rumpfs zeigten. Die Körperteile, die mit den Genitalien ko-repräsentiert sind, sind solche, die während der Kopulation von Männchen und Weibchen in Berührung kommen. Im Gegensatz zum physiologischem Dimorphismus, zeigten Cytochrom Oxidase Färbungen von Schicht 4 einen frappierenden Monomorphismus von kortikaler Penis und Klitoris Repräsentation. Dies ist ein überraschendes Ergebnis, wenn man den Dimorphismus der externen Genitalien bedenkt. Zusätzlich haben wir ein massives Wachstum der relativen Größe des Genital Kortex während der Pubertät gefunden. Unsere Ergebnisse liefern Einsichten, in die bisher wenig erforschte Repräsentation von sexueller Informationen in der Hirnrinde.

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Outline of the thesis

Chapter 1 briefly introduces social touch in rats, with a focus on facial and genital touch. Further it gives a short overview of brain body maps and sensory touch processing. In addition, the motivation of the conducted work is described.

Chapter 2 summarizes the methods used to perform the described studies.

Chapter 3 describes the impact of social facial touch on membrane potential dynamics (V_m) in barrel cortex neurons.

Chapter 4 investigates the cortical representation of genitals in male and female rats.

Finally, Chapter 5 discusses the main findings and contains a brief overall conclusion.

Chapter 1

General Introduction

How does the brain control action? Which neural structures are responsible for a given behavior? These are central and important questions in the field of neuroscience. The part of the brain that is responsible for processing sensory information has been long known and is called the neocortex. It contains primary sensory areas which represent low level stimulus information. Somatosensory information, such as touch, is processed in the primary somatosensory cortex (S1) of mammals. Touch is essential during social interactions of both, humans and animals. Therefore it seems likely that tactile stimuli carry meaningful information in a social context. Thus they may be processed differentially in S1 as compared to touch of objects.

Rats are highly social animals showing a great amount of bodily contact. Hence we chose to investigate how social touch is processed in rodent S1. More precisely, this thesis focuses on two forms of touch which occur during multiple social behaviors of the rat and are therefore likely to be of biological relevance: social facial touch and genital touch. The results of the thesis contribute greatly to a better understanding of the role of primary sensory areas during social and biological relevant behaviors.

1.1 Social touch in rats

Rats are very social animals. Their natural environment mostly consists of burrows and tunnels which are shared as housing among conspecifics. Rats' social repertoire is broad and can be divided up into the categories of aggression/dominance, mating/parental care and play behavior (Barnett, 1958). All of these behaviors share an important similarity, namely they all involve some form of touch. Social touch is not only used by rats but it is also essential among monkeys and humans. It was shown that grooming behavior in monkeys promotes pair bonding which has an important impact on their reproductive fitness (Dunbar 2010). Among humans, skin-to-skin contact between a mother and her low birth weight infant decreased the mortality rate and the risk of severe infection or sepsis (Conde-Agudelo and Díaz-Rossello 2014). The quality of maternal care in rodents appears to shape stress responses in adult offspring (Menard et al. 2004).

In rats, the specific behavioral patterns of maternal care, play behavior, mating or aggression have been studied in wild (Barnett 1958) and laboratory rats (Barnett 1963). While laboratory rats do not actively choose to live in groups, it is known that isolation results in social deficits (Hall 1998, Hol et al. 1999). Social encounters occur frequently in co-housed unisex groups and usually start with investigatory behavior (Nomoto and Lima 2015). When tracking this investigatory behavior in a pair of two rats under an intruder paradigm (Fig. 1.1A), two types of somatosensory interactions can be observed: social facial touch and anogenital sniffing (Fig. 1.1B; Wolfe et al. 2011). Anogenital sniffing is the most frequent behavior in the first minutes of investigation, indicating that the touch and the smell of the anus and the genitals are of great importance for the animals. With time, genital touch decreases and facial touch with the whiskers on the animal's snout increases (Fig. 1.1B).

When a male and a female rat are allowed to freely interact, copulatory behavior is often observed. However this strongly depends on whether the female rat is sexual receptive (in the estrus phase of the sexual cycle) or not (in the non-estrus phase of the sexual cycle). Facial touch and genital touch are prominent behavioral features during copulatory behavior. During other social behaviors, such as aggression and dominance or parental care, facial and genital touch are also important attributes to transmit the sexual and emotional state of an animal. Hence, social facial and genital touch stimuli are studied in this thesis and we will elaborate more on these forms of touch in the following sections.

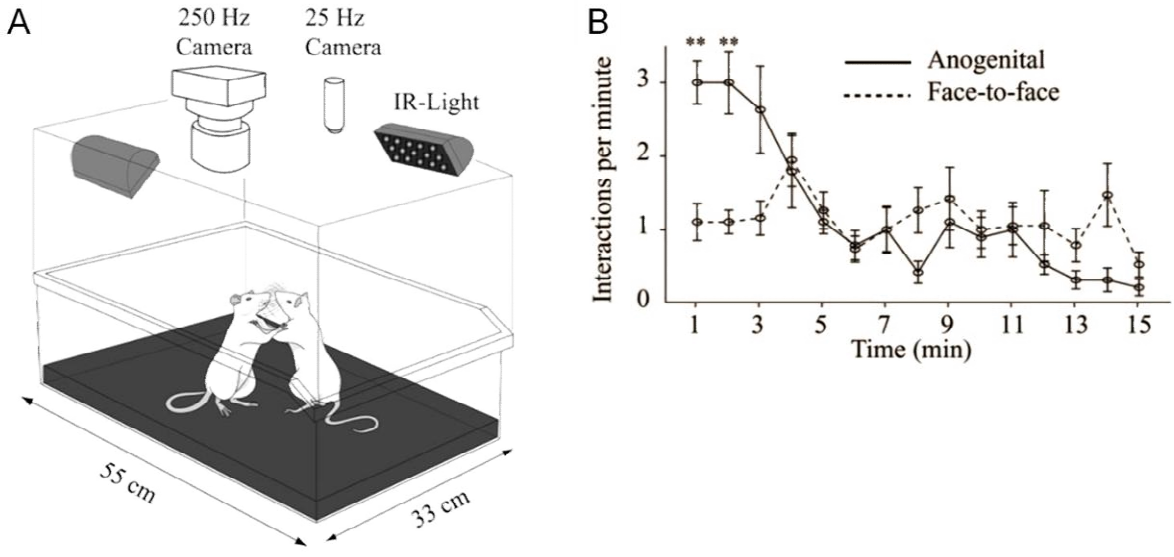

Figure 1.1: Social investigatory behavior in an intruder experiment. (A) Experimental setup: In the intruder paradigm, an intruder rat was placed in the home cage of a second rat. Animals were allowed to interact for 15 min and were filmed with both, high- (250 Hz) and low-speed (25

Hz) cameras. **(B)** Anogenital sniffing occurred significantly ($p<0.01$, Wilcoxon's rank sum test) more during the first 2 min (n=19 trials each, five pairs of rats). All error bars are standard errors. Figure adapted from Wolfe et al. 2011.

1.1.1 Social facial touch

Social facial touch occurs throughout the animal kingdom. Sea lions use vibrissal touch during close interactions (Peterson and Batholomew, 1967) and monkeys express a face-to-face kissing-like behavior when reconciling after fights (de Waal, 2000). In rats, facial touch is involved in the transmission of food preferences (Galef 1986) and in the communication of aggressive behaviors (Ahl 1986, Wolfe et al. 2011). A study by Wolfe and colleagues (2011) quantified social facial touch by putting a pair of rats onto an elevated platform, which was interrupted by a gap between the rats (Fig. 1.2A). Social facial touch was observed voluntarily and frequently (Fig. 1.2B). While approaching each other, whisking amplitude decreased and whiskers were held more protracted, presumably to get the maximal sensory input from the conspecifics whiskers. Interestingly, facial touch in rats seems to be sexually dimorphic. Male rats hold their whiskers more protracted, but show no difference in whisking pattern while interacting with either another male or a female rat. In contrast, females whisk with smaller amplitudes onto males compared to females, suggesting a preference for tactile stimulation by males (Wolfe et al. 2011).

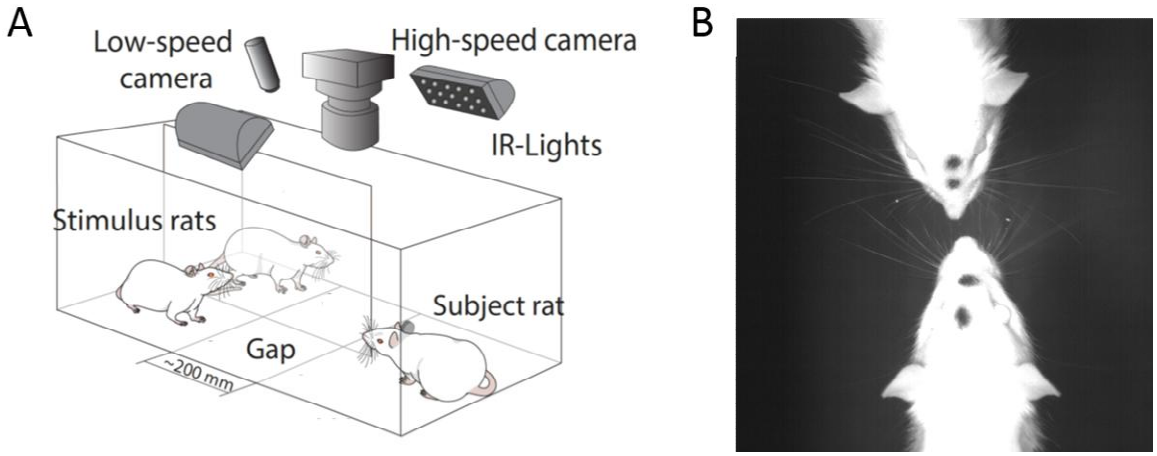

Figure 1.2 Social facial touch in a gap-paradigm. (A) Two elevated platforms were separated via a gap. A subject rat was put on one platform and could interact with different stimulus rats placed on the platform vis-à-vis. **(B)** Social facial touch was filmed and quantified by low- and high speed videography. Figure modified from Wolfe et al. 2011.

Rats voluntarily engage in social facial touch once they are restricted to this form of interaction via a gap. This points to a role of facial touch in the communication of other social modalities. Because of the multisensory nature of a social interaction (Brecht and Freiwald 2012), it is challenging to identify which form of sensory cue is most important for the behavioral outcome. Rats exchange auditory cues (by emitting ultrasonic calls), tactile cues (by touching each other), olfactory cues (by the release of pheromones associated with endocrine release in females) and visual cues. Hence, information about the sex and the sexual or emotional status of an animal may be transmitted by an ensemble of at least two of the above mentioned sensory stimuli (Barnett 1963). An early study showed that a male rat can distinguish the odor of a female from that of a male, and the odor of an estrus female from that of another non-receptive female. However, copulatory behavior whereby the male mounts a sexual receptive (estrus) female, still occurs in anosmic males. Combined blocking of olfactory signaling and a second sensory modality (vision or somtaosensation) results in copulatory deficits (Magnen 1951). These results support the idea of multisensory integration during many social behaviors.

1.1.2 Genital touch

Anogenital touch is observed during multiple social touch behaviors. Tactile stimulation of the pup's anogential region by licking by the mother is important for the development of proper urination and defecation (Friedman et al. 1981, Gubernick and Albert 1985). In addition, the development of sexual behavior is affected when the frequency of anogenital licking is decreased. Low licked pups showed longer ejaculatory latencies, longer post-ejaculatory intromission latencies, and longer inter-intromission intervals (Moore 1984). Further, anogenital sniffing is involved during sexual behavior and, more precisely, during the pre-copulatory period. The pre-copulatory period describes the behavioral sequence before two rats copulate, hence before the male mounts the female and performs successful intromissions and finally ejaculation (Barnett 1963). Anogenital touch consists of active sniffing and licking of the other rat's genitalia which is accompanied by a characteristic posture with the head pointed downwards (Barnett 1963, Fig.1.3).

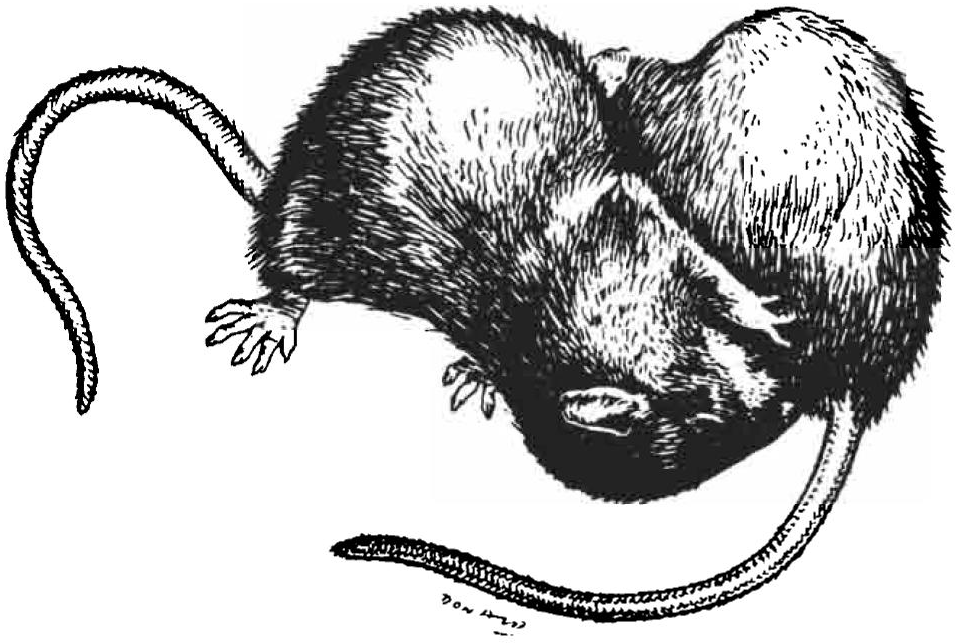

Figure 1.3: Anogenital sniffing. In the first minutes of social investigatory behavior, licking and sniffing occurs predominantly. The posture during anogenital sniffing is characterized by the head pointed downwards. Adapted from Barnett 1963. The rat: A study in behavior, p.109.

As described above, anogenital sniffing is the most prominent behavior in the first minutes of a social interaction, preceding facial encounters. Communication with the conspecific via facial touch may commence once the animal has gained maximal olfactory input from the other animal genitals (Wolfe et al. 2011). When a male encounters a female he will express the above mentioned pre-copulatory behavior, including anogenital sniffing, licking and chasing the female. A female rat in a sexual non-receptive state (estrus) will reject and kick off the male when he attempts to mount her. In contrast, a female in estrus (sexual receptive state) will allow mounting before expressing typical pre-copulatory behaviors, such as actively sniffing the male body and genitalia before briefly running away and pausing. This so called darting behavior is accompanied by characteristic ear-wiggling (Barnett 1963). While the male follows the female and starts to mount her, the female will display a typical posture, which allows intromission by the male. The lordosis position is characterized by her head facing upward and the coccygeal region lowered downward (Fig.1.4).

Figure 1.4: Rat mating behavior. The female is displaying the so called lordosis position during which the coccyx is raised and her spine deflected. Thereby intromission by the male is possible. The male is grasping the female flanks with the forelimbs. Adapted from Barnett 1963. The rat: A study in behavior, p. 111.

Taken together facial touch and genital touch are two important social behavioral events by which rats can communicate their emotional status to a conspecific, may it be aggressive, playful or sexual receptive. Sensory information received by these body-parts (whiskers and genitals) might, therefore, carry more than neutral tactile information.

1.2 Somatosensory cortex: from humans to rats

Somatosensory information, such as touch, is processed in the S1 of mammals. Penfield and colleagues performed pioneering mapping studies on human somatosensory cortex in the beginning of the twentieth century. Applying electrical stimulation to the S1, they were able to map the different body parts onto the somatosensory cortex in the brain. Surprisingly, Penfield and colleagues found, that the actual size of a body part was not proportional to the share of S1 dedicated to its neural representation. Instead the size of cortical representation closely correlated with the tactile sensitivity of the specific body part. While the representations of lips, tongue and hands take up large parts of the S1, less sensitive regions such as the trunk, arms and legs occupy comparatively small cortical areas. The same experiments were performed in the primary motor cortex. By doing, so Penfield generated the first somatotopic maps known as the sensory (Fig. 1.5 left) and motor homunculus (Fig. 1.5 right).

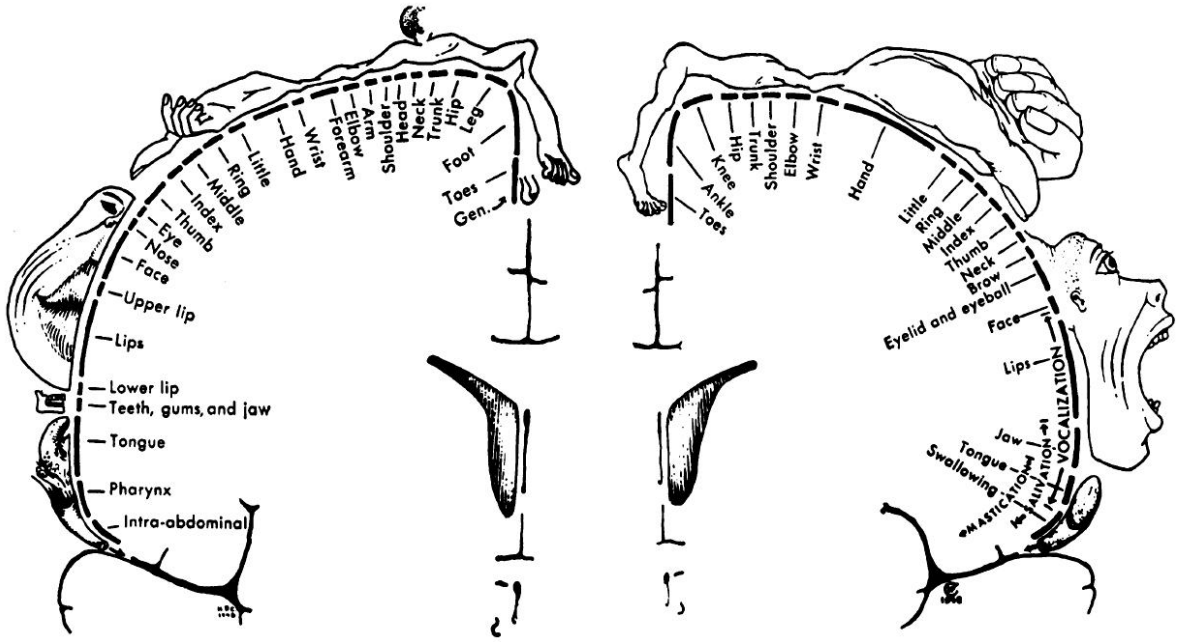

Figure 1.5: Penfield's homunculi
Left side: Body map based on touch and related sensations named sensory homunculus. On the right side the mirror picture is representing the primary motor area, a basic map for voluntary movements (motor homunculus). Adapted from: Penfield and Rasmussen (1950). The cerebral cortex of man. Copyright 1950 Macmillan Publishing Company; copyright renewed 1978 Theodore Rasmussen.

In rats, two early studies attempting to generate body maps were done by Woolsey and Van der Loos (1970) and Welker (1971). The main finding of the first study was the discovery of an anatomical barrel map (Fig. 1.7). In humans the tongue and lip claim a huge representation of the somatosensory cortex, whereas in rats the whiskers on the rat's snout (Fig. 1.6) have the largest relative representation in rat S1. Each whisker on the rats face is represented by a single barrel in the brain (Fig.1.7, inset). Because rats are nocturnal animals, their perception of the environment heavily relays on scanning it with their whiskers. Hence the underlying anatomy of somatosensory cortex, namely the large whisker representation, exquisitely reflects the sensory periphery.

Figure 1.6: Photograph from the study by Welker (1964). A rat's face from the front is shown. Note the remarkable whisker or vibrissae on the rat's snout which they use to sense obstacles, textures or conspecifics in their environment. Adapted from Welker 1964.

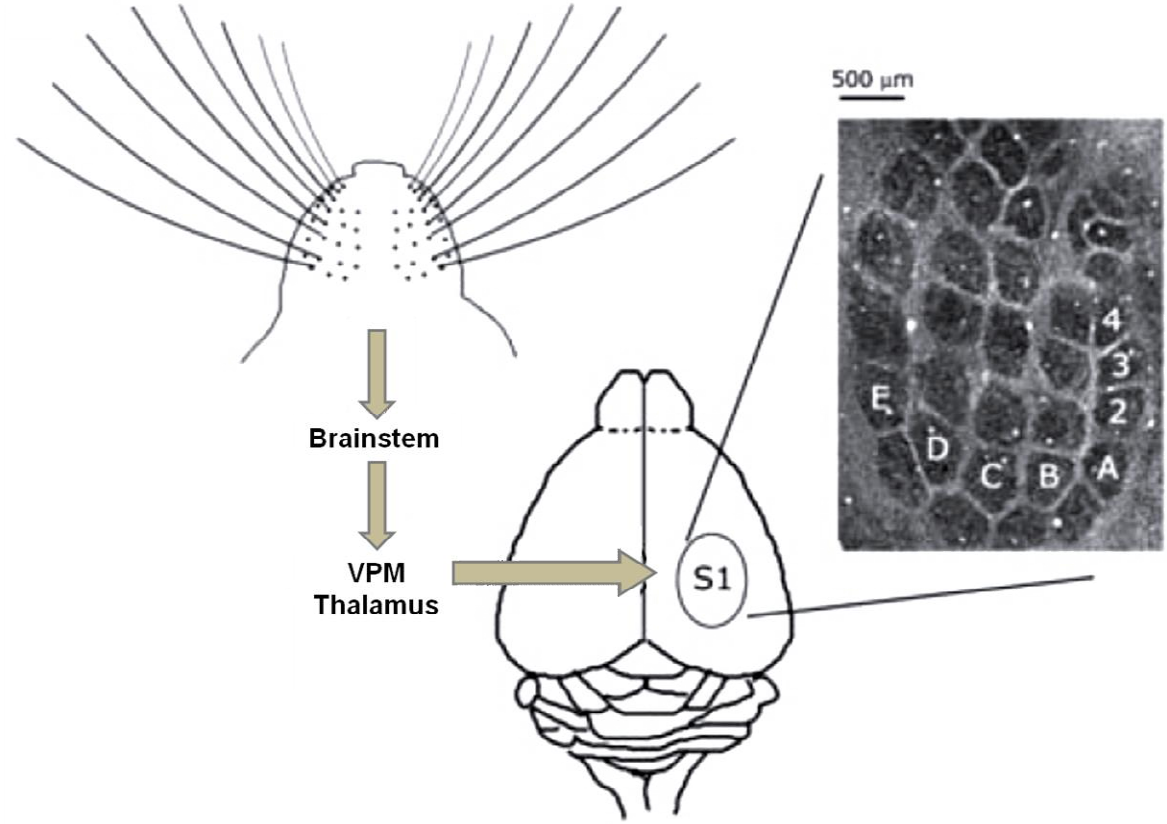

Figure 1.7: The whisker-to-barrel system. Incoming information from the whiskers travels via the brainstem to the thalamus and enters primary somatosensory cortex (S1). Inset shows a Cytochrome oxidase stain of the barrels in S1. Figure adapted from Feldman and Brecht (2005).

1.3 Touch processing in the rat somatosensory cortex

Sensory signals detected by the whiskers are relayed via the brain stem to the S1. Specifically, whisker follicles located in the skin of the rat's snout are innervated by the trigeminal nerve of the trigeminal ganglion, which sends it to different brain stem nuclei. Finally it enters the somatosensory cortex via the lemniscal (see Fig. 1.7), paralemniscal and extralemniscal pathway (reviewed in Lübke and Feldmeyer 2007). In the lemniscal pathway, information is relayed from the whisker pad via the principle trigeminal nucleus to the dorsal medial region of the ventroposteriormedial nucleus (VPm). From here it is sent mainly to cortical layer 4 and to a lesser extent to layers 3, 5B and 6. In the paralemniscal pathway sensory signals travel from the interpolar spinal trigeminal nucleus to the posteromedial nucleus (POm) of the thalamus from where they are mainly relayed to neurons in layer 1 and 5A of the cortex. In the extralemniscal pathway, sensory signals are sent from the interpolar spinal trigeminal nucleus to dysgranular regions of S1 (named septa). Nerve cells in the leminiscal pathway have small receptive fields and provide fast temporal signals, whereas those in the paralemniscal and extralemniscal have large receptive fields and provide slow input (reviewed in Lübke and Feldmeyer 2007).

Many studies focused on the activity in somatosensory cortices during active touch. However, these studies were mostly conducted under precisely controlled conditions and involve the presentation of an object (Knutsen et al. 2006, O'Connor et al. 2010) or a texture to a head-fixed animal. In their natural environment however, rats not only have to identify objects and obstacles, they also encounter conspecifics. Natural occurring social behaviors, such as the interaction between mother and offspring or sexual contact between female and male are important for the survival of the animal and involve multisensory stimuli (Brecht and Freiwald 2012). Therefore it is important to investigate whether sensory stimulation elicited during social interactions are processed differently in the brain. Studies that investigate how socially meaningful stimuli are processed in primary sensory areas are rare and were mostly conducted using fMRI (functional Magnetic Resonance Imaging) in humans (Bufalari et al. 2007, Bufalari et al. 2014, Gazzola et al. 2012). Gazzola et al., for example, showed that activity of somatosensory cortex depends on the perceived gender when test subjects were gently touched by the experimenter (Gazzola et al. 2012). However these investigations suffer from a number of limitations, including the coarse spatial resolution of the imaging data, the simple experimental setup and the difficulty to isolate the social component of somatosensory stimulation. Nevertheless the results suggest, that the role of primary cortices in processing the social

context of somatosensory stimulation arising during the interaction with a conspecific, might have been underestimated.

Electrophysiological recordings in freely behaving and interacting animals are technically challenging. Many studies on social behavior target the hypothalamus (Nomoto and Lima 2015, Scott et al. 2015) or the medial amygdala (Bergan et al. 2014) rather than cortical circuits. Few studies examined cortical activity in rats, which strengthen the proposition of different neural responses in primary cortices (Bobrov et al. 2014, Rao et al. 2014) during social touch behavior. In a study by Bobrov et al. (2014), extracellular recordings were obtained from barrel cortex neurons while animals freely engaged in social facial touch. Interestingly, neurons recorded from female barrel cortex showed a slightly higher baseline firing activity compared to those recorded from the barrel cortex in males. The overall response rates to social touch in male barrel cortex were strong and the same regardless of the interaction partner's sex. Single units recorded in females, however, were more weakly modulated by social touch and depended more strongly on the sex of the interaction partner as well as on the sexual state of the recorded animal. If the females were non-receptive (meaning in the stage of non-estrus), barrel cortex neurons responded equally well during interactions with males and females. During estrus, in which the animal is supposedly sexual receptive, single unit recordings were mostly inhibited during facial interaction with a female and overall excited (although sparsely) when animals interacted with a male. Bobrov et al. (2014) explored how activity in S1 is modulated in a social setting (i.e. freely moving animals interacting with each other). In line with the human fMRI experiments by Gazzola et al. (2012) these results confirm that the role of primary somatosensory cortex in affective processing of social touch is underestimated (Gazzola et al. 2012).

Bobrov et al. (2014) used extracellular recordings to monitor the output signals of the neurons, in the form of spikes. The full resolution of inputs to the cell, however, can only be captured using intracellular recordings (Chorev et al. 2009). Further, intracellular recordings allow to visualize the cellular morphology and to monitor intrinsic properties. Since the sexually differential responses reported by Bobrov et al. (2014) could not be explained by the mechanics of interaction patterns, it is worthwhile exploring whether neurons recorded in males and females show different intrinsic properties or thresholds to spiking. Another open question is, if the oscillation in spike trains were correlated to the animal's whisking or if barrel cortex neurons spike during a specific phase of whisking.

The cellular membrane potential (V_m) was shown to be strongly locked during free, exploratory whisking (Crochet & Petersen 2006, Poulet & Petersen 2008, Crochet et al. 2011). How subthreshold activity is altered during whisking in a social context (i.e. social facial touch) has not been explored yet. To answer this question, the first study of this thesis (Chapter 3) explores the impact of social facial touch on the V_m dynamics in barrel cortex neurons. Since whole-cell patch clamp recordings are extremely challenging in freely-moving animals (Chorev et al. 2009), the experiment was designed such that the subject rat was head-fixed, while the interaction partner (the stimulus rat) was presented on the experimenter's hand.

1.4 Genital representation in the brain

How touch is processed in the barrel cortex has been studied in detail. There are even a few studies investigating the role of social facial touch in rats and how this meaningful form of touch modulates S1 activity (Wolfe et al. 2011, Bobrov et al. 2014). Another form of social touch is genital touch, which has been investigated mainly during sexual behavior. Sexual behavior and the underlying neural circuits have been extensively studied in rodents (Bergan et al. 2014, Lee et al. 2014, Nomoto and Lima 2015). However, how sensory stimulation of the genitals is represented in the cortex has somehow been largely ignored.

While body parts, which are involved during facial touch (i.e. the whiskers of the rat's face and the mouth and hands in humans) are overrepresented in size in the somatosensory cortex of humans and rodents, one wonders where and how the sensitivity of the genitals is represented. Returning to early work on the rat homunculus it is surprising that genitals do not appear in the body maps (Welker 1976, Fig. 1.8A; Chapin and Lin 1984, Fig. 1.8B). A close examination of the famous Penfield & Rasmussen map (Fig. 1.8C), depicted in many textbooks, shows that genitals are illustrated by a non-erect penis along a scrotum on the mesial cortical surface below the foot of the somatosensory homunculus. A more recent imaging paper (Fig. 1.8D, Kell et al. 2005) illustrates penis sensations on the lateral cortical surface between the legs of the somatosensory homunculus (i.e. at a somatotopically appropriate position). Regarding sexual dimorphism, these studies do not provide an answer. Welker (1976) only used female rats while Chapin and Lin (1984) only used male rats. Similarly the Penfield and Rasmussen homunculus only depicts a penis and scrotum but no vagina and clitoris, generally largely neglecting females in their work. Whether this is due to a lack of data on woman, to a lack of knowledge concerning

the menstrual cycle or to plain historical issues of propriety remains unsolved (Di Noto 2013). Subsequent mapping studies in women identified genital representations, although their position in the somatosensory cortex vary (reviewed in Di Noto 2013). Another issue that has been ignored so far with respect to female genital representation is the estrus cycle. It is known that ovarian steroids influence the activity of many brain areas, such as the hippocampus or the hypothalamus (Woolley 2007). More importantly barrel cortex activity is modulated by the animal's sexual receptivity (Bobrov et al. 2014). Those studies raise the question of whether the response patterns described by Welker (1976) might be variable given the fact that hormonal fluctuations were not considered.

Taken together, there is no consensus regarding the cortical representation of genitals in humans while there is a total lack of knowledge about genital representations in the rat. Therefore we wondered, what a high-resolution map of genital representations in rats may look like and what it might tell us about the sexual differentiation of the mammalian brain. We combined physiological and anatomical mapping of cortical layer 4 in order to investigate the somatosensory representation of the genitals. Further the study aimed at characterizing how simple tactile stimuli are processed in the genital cortex of male and female rats. In this way the question if genital touch is not only relevant on the behavioral level, but may also modulate somatosensory cortex in a differential way, should be approached.

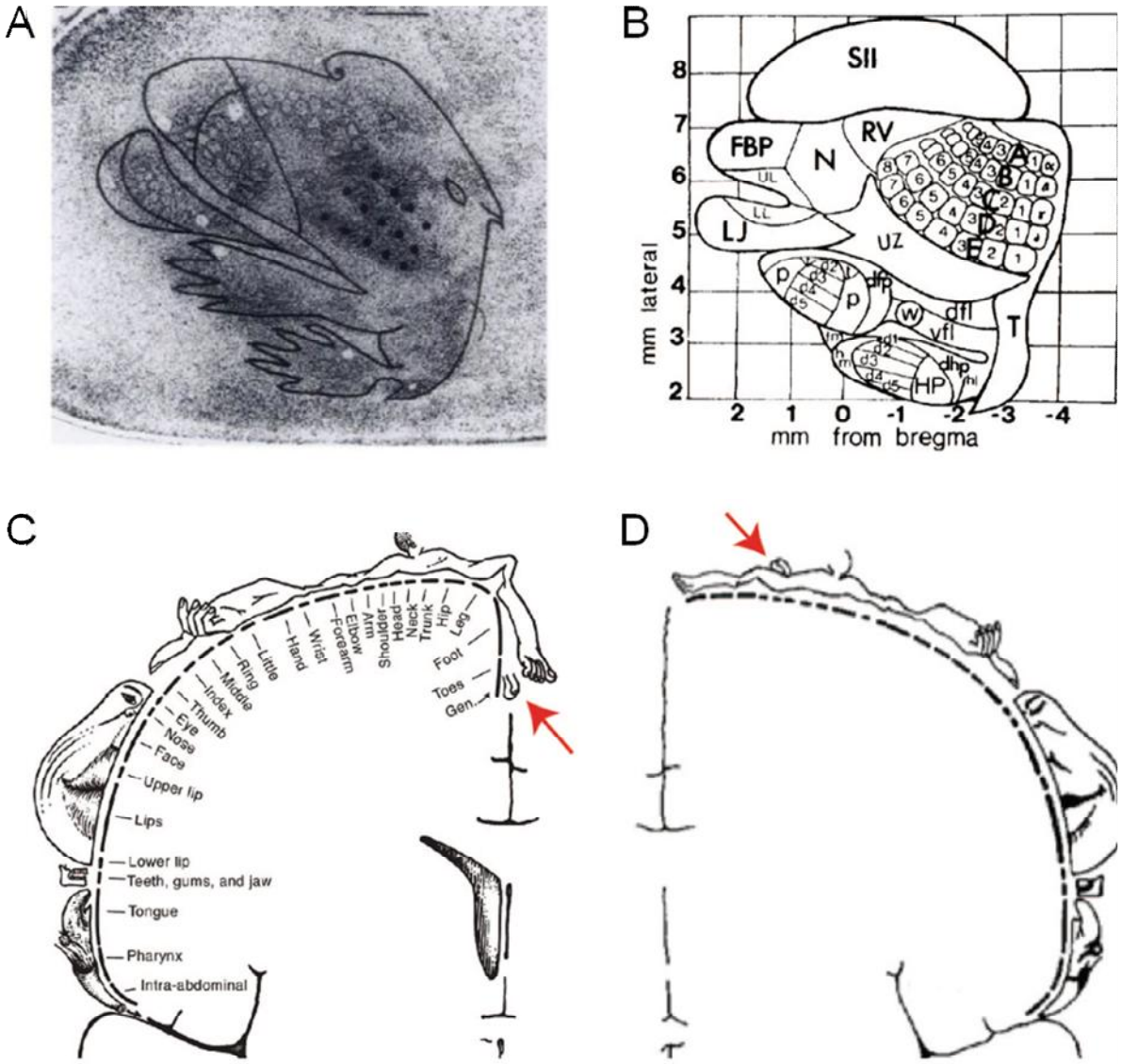

Figure 1.8: Absent and symbolic genitals in somatosensory cortex. A, B, Absent genitals. Rat work by Welker (1976), and the widely used detailed map provided by Chapin & Lin (1984). Both did not report genitals and in Chapin and Lin, genital cortex was assigned as vfl (ventral forelimb). Abbreviations: T, trunk; hl, hindlimb; HP, hindpaw; dhp, dorsal hindpaw; d, digits; hm, hindlimb muscle; vfl, ventral forclimb; dfl, dorsal forelimb; w, wrist whiskers; dfp, dorsal forepaw; p, palm; d2-5, digits 2-5 of forepaw; t, thumb (pollux); UZ, unresponsive zone; A-E, whisker rows 1-8, numbers stand for arcs of the whiskers; RV, rostral small whiskers.: N, nose; FBP, frontobuccal pads; UL, upper lip; LL, lower lip; LJ, lower jaw. **C, D,** Symbolic genitals. The classic Penfield & Rasmussen map of human somatosensory cortex (**C**). The genital representation (red arrow) is based on a few cases of intrasurgical surface stimulation of somatosensory cortex. Only males are depicted and the mapping resolution is in the several mm range, hence providing no genital details. The genital illustration is small and 'symbolic'. **D,** Recent functional magnetic resonance imaging (resolution ~several mm) of penis sensation (red arrow, Kell et al. 2005). No genital details were resolved and the illustration is small and 'symbolic'.
Figure A adapted from Welker 1976, Figure B adapted from Chapin and Lin 1984. Figure C was adapted from Penfield and Ramussen 1950 and Figure D from Kell et al. 2005.

Chapter 2

Methods

2.1 Animals

Long-Evans rats were provided by the Marine Biological Institute, Woods Hole (USA) and Wistar rats were obtained from Harlan (Horst, The Netherlands). All animals were housed in a group of at least two rats and maintained on a 12 hours light / 12 hours dark cycle with food and water ad libitum. After surgery subject rats were removed from the group and single housed. Stimulus rats were adult (P50-P140) and housed in same sex pairs. They were initially handled in their cages before habituating them to the transport cages and the setup.

All experimental procedures were performed according to American and German guidelines on animal welfare under the supervision of local ethics committees

2.2 Surgeries

2.2.1 Surgeries for acute and chronic whole-cell patch clamp recordings (chapter 3)

For surgery, animals were anesthetized by injection of an initial dose of 100 mg/kg ketamine and 7.5 mg/kg xylazine. Respiration, blink and pinch reflexes were observed throughout the surgery and, if needed, animals were injected with an extra dose (25%) of ketamine/xylazine mixture or a 25% dose of ketamine alone. Body temperature was monitored using a rectal probe and maintained with a heating pad (Stoelting) set to 34-36°C. Lidocaine was locally injected in the scalp, which was then removed. During the first surgery a metal bolt was implanted on the skull of the animals head using an UV hardening glue (Kerr) and dental cement (Heraeus). At the time of surgery subject rats were between 25 and 27 days of age. After recovery rats were habituated to the head-fixation procedure described below. After successful habituation subject rats underwent a second surgery during which a craniotomy for patch clamp recordings was made. To this end a 1 to 1.5mm wide whole was drilled over the barrel cortex. The position of the craniotomy was 5.5mm posterior and 2.5mm lateral to bregma. The dura was removed using a bent needle. The preparation was protected by implanting a threaded plastic chamber with a lid, which surrounded the craniotomy.

Recording experiments commenced after rats had recovered from surgery (P30-P34). Between recording sessions the preparation was covered using silicone (Kwik-Cast, World Precision Instruments).

In the acute patching experiments presented in chapter 3, subject rats were aged between P25 and P35. Animals were anaesthetized with urethane (1.5-2 g/kg). The parameters and size of craniotomy were the same as mentioned above.

2.2.2 Surgery for the acute mapping experiments (chapter 4)

For mapping experiments (n = 11, 6 males and 5 females) and the single-unit stimulation recordings (both chapter 4, n = 8, 5 males and 3 females) Long-Evans and Wistar rats (P22–P30, n = 11, 6 males and 5 females) were anesthetized using urethane (1.4 g/kg, i.p.). After putting the animals in a stereotactic frame, a whole was cut into the scalp, and tissue and fat was removed. Afterwards an approximately 5 x 5 mm sized craniotomy was made over the area of somatosensory cortex (5 mm posterior to and 5 mm lateral to bregma).

2.4 Habituation procedure (chapter 3)

Following the first surgery, animals were allowed to recover overnight. On the next day habituation to the head-fixation commenced. Rats underwent three habituation sessions per day, starting with 5 minute period of head-fixation which was incrementally increased by 10 minutes per session. The habituation period depended on the animal's behavior but was typically 2-4 days long. During habituation sessions, animals were gradually exposed to the experimental procedures and environment encountered during subsequent recordings. This included light, noise from micromanipulators and touching of the implant. In the beginning of the study, stimulus rats were not introduced to the subject rat during habituation sessions in order to test whether novelty affected the observed responses. We later determined that responses were the same regardless of stimulus rat novelty. In later habituation sessions, stimulus rats and objects were introduced to the subject rat in order to reduce stress at the time of recording experiments.

2.5 Social facial touch behavior in head-fixed animals (chapter 3)

Social interactions were staged by the experimenter by presenting a stimulus rat to the head-fixed subject rat (Fig. 3.1A). The subject rat was either awake (chronic patching experiments) or anaesthetized (acute patching experiments). An infrared (IR) light (TV6830, wavelength 880 nm, Abus, Wetter, Germany) was installed together with a 25Hz low-speed (K240, Siemens, München, Germany) and a 250Hz high-speed camera (A504k, Basler, Ahrensburg, Germany) above the experimental setup. Behavior of the subject rat was monitored throughout the recording session using low-speed videography. The high-speed camera was turned on when the social interactions took place. A social interaction (also referred to as an 'episode') was defined as the two rats touching each other with their whiskers. The beginning of an interaction was set to the first whisker overlap, and the end of an interaction was the time-point at the end of whisker overlap. These time settings were determined using low- and/or high-speed videos. The subject and stimulus rats were able to see each other while approaching. In addition, two other behavioral events were detected by videography: the pre-contact period (beginning with the approach of the stimulus rat and lasting until the first whisker overlap) and the post-contact period (starting from the last whisker overlap and ending when the stimulus rat was out of sight).

2.6 Electrophysiology

2.6.1 Whole-cell Patch clamp recordings in head-restrained rats (chapter 3)

After the second surgery, animals were allowed to recover for 3-4 hours. After full recovery, subject rats were put in a head-fixation frame for recording sessions. If no whole-cell recording was obtained during the first day (day of second surgery), recording sessions continued for the two following days. Patch pipettes were lowered into the cortex with positive pressure (200-300 bar). When the pipette reached 150-200 μm below the surface, the pressure was lowered to 30 bars to search for cells. When the pipette resistance increased, suction was applied to establish a gigaohm seal and finally the whole-cell configuration. Whole-cell patch-clamp recordings were made using glass electrodes made of borosilicate glass tubes (Hilgenberg, Malsfeld, Germany). The resistance of the patch pipettes ranged from 4 to 6 MΩ. Pipettes were filled with an internal solution containing the following (in mM): K-gluconate 130, Na-gluconate 10, HEPES 10, phosphocreatine 10, MgATP 4, GTP 0.3, NaCl 4 and biocytin (~0.05%), pH 7.2. Recordings were amplified by an amplifier (Dagan, Minneapolis, USA), filtered at

3–10 kHz and sampled at 50kHz by a data-acquisition interface (LIH 1600, HEKA, Lambrecht, Germany) controlled by the Patchmaster software (HEKA). Firing pattern, IV (current voltage) curves and spontaneous activity were recorded using custom-written protocols in the recording software (Patchmaster). Input resistance was computed offline. Recordings were exported and analyzed in Matlab 2014a (Natick, Massachusetts, USA).

2.6.2 Receptive field mapping, whole-cell and single-unit recording (chapter 4)

All extracellular recordings and hand mapping of receptive fields were performed in the left hemisphere of 6 males and 5 females with a 1 MΩ sized glass electrode. Voltage signals were amplified, differentially filtered for spikes, and sent to an audio monitor using a patch-clamp amplifier (Dagan) in current-clamp mode. An easy access to the genital region of the animals was guaranteed by the following points: (1) The stereotaxic frame was close to the opening of the Faraday cage. (2) The animals head faced rightwards; thus the right body side (i.e. the side contralateral to the left hemisphere, which we mapped) was facing the experimenter. (3) The animals were elevated by a supporting plastic brick putted below the anterior body half. In this way posterior parts of the trunk were accessible from all sides while anterior ventral parts of the trunk were less well accessible.

For cortical mapping of the somatosensory cortex, we searched for clear tactile responses at each recording site. Recording electrodes were lowered in the cortex between deeper layer 3 (600 μm) and upper layer 5 (1300 μm). Clear tactile responses were tested while palpating different body parts of the animals. In this way receptive fields could be determined for every electrode position. Before the first penetration, the recording pipette was set to 0 at the point of bregma using micromanipulators (Luigs & Neumann, Ratingen, Germany). The first penetration was made at 0.5 mm posterior and 2.5 mm lateral to bregma. In the following the recording pipette was moved in 0.5 mm steps over the craniotomy until the full size of the craniotomy was penetrated. Once tactile responses to genitals were encountered, we reduced the penetration spacing to 0.25 mm steps in order to improve mapping precision for the cortical genital area. Receptive fields were hand-plotted by systematically palpating the animal's body surface. Stimulation of the body surface was done in two different ways. For all body parts beside the vulva we used a short metal bar with which we applied fast gentle (resulting only in little skin indentation) strokes. The vulva was also stimulated with a thin (1 mm diameter) metal wire, with which we systematically stimulated internal parts of the vulva/clitoris in

females. In all receptive fields close to the animal's midline, we performed bilateral stimulation of the respective skin areas. Bilateral (midline-crossing) receptive fields were rare, however, and purely ipsilateral receptive fields were not observed.

For single-unit recordings we used 5 Mega Ohm glass pipettes and recorded large (> 0.5 mV) spikes of individual cells in the juxtacellular configuration on a Dagan-amplifier. A juxtacellular recording was obtained by injecting positive current pulses through the pipette while at the same time slowly stepping (one step 3µm) through the brain. This procedure is called the Pinault protocol and is described in detail in Pinault 1996.

Once spike were detected and the recording was stable, spontaneous spiking activity was recorded before stimulating different body parts. For air-puff stimulation a pointing cone was positioned on the body part to be tested. Air-puffs were applied for 1 ms every 2 seconds with a pressure of 1 mbar. If the recording was stable and of sufficient length the following body parts were stimulated with an air-puff: genitals (clitoris, vulva and penis, scrotum), trunk and forearm. These juxtacellular recordings were performed in 4 male and 3 female prepubescent rats. Recording traces were exported and analyzed in Spike2 (CED, Cambridge, UK) and Matlab 2014a (Natick, Massachusetts, USA).

Whole-cell patch clamp recordings were obtained in the same way as described for the chronic experiments (see under 2.6.1). When a stable recording was established, air-puff stimulation was applied to the different body parts (as described above).

2.7 Histology

After all successful recordings (receptive field mapping, whole-cell patch clamp and juxtacellular experiments), animals were anaesthetized using a mix of ketamine and xylazine or urethane (20% solution) and perfused with phosphate buffer followed by a 4% paraformaldehyde solution (PFA). Brains were stored overnight in 4% PFA. During the chronic and acute whole cell patch clamp recordings (chapter 3), the internal pipette solution contained biocytin for post-hoc analysis of the recorded cells. After post-fixation, the brains were cut in 150 µm coronal sections. Sections were then stained with cytochrome oxidase (protocol Wong-Riley 1979) and the biocytin-filled neurons were visualized using the avidin-biotin-peroxidase method.

After successful physiological mapping, juxtacellular or whole cell patch clamp recordings (chapter 4), animals were perfused transcardially in the same way as above. For the analysis of anatomical maps, the procedure of perfusion was the same. Afterwards brains were removed and hemispheres were separated. After removal of

subcortical structures cortical hemispheres were flattened between two glass slides separated by clay spacers. Glass slides were then weighed down with small ceramic weights for ~3 h. Flattened cortices were removed from the glass slides and stored overnight in 2% PFA. On the next day 100 µm sections were cut on a Vibratome (Leica, Wetzlar, Germany). A cytochrome oxidase stain was performed according to the protocol of Wong-Riley (1979). After mounting the sections on microscope slides, images were taken on a Leica M165 FC microscope. Outlines of granular somatosensory regions (indicated by a dark precipitate from the cytochrome oxidase stain) were drawn using ImageJ software.

2.8 High-speed videography and tracking (chapter 3)

Prior to recording sessions, whiskers of subject and stimulus rats were tagged. To this purpose animals were anaesthetized using isoflurane and anesthesia was sustained by applying isoflurane continuously through a mask covering the rats' mouth and nose. The C2 whisker was tagged by forming a small spherical drop (0.5 – 1.0 mm diameter) of high-viscosity ultraviolet sensitive epoxy (3021 UV-adhesive, DYMAX, Torrington, USA) on the whisker. After hardening, the bottom half of the epoxy sphere was covered with silver paint and the tag was additionally fixed on the whisker with a small drop of superglue. Upon illumination from above this created a very bright image (Wolfe et al. 2011, Figure 1). Social interactions were monitored using a high-speed camera installed above the setup. High-speed acquisition was performed at 250 Hz with 1280 x 1024 pixels and controlled by custom-written LabView programs (National Instruments, Austin, Texas, USA). Before presenting the stimulus rat to the head-restrained subject rat, high-speed video acquisition was manually started. Whisker tracking was done offline using a custom-written code in Matlab. Head, nose, whisker base and tag positions were set manually, whereas the contour of subject and stimulus rat was computed automatically. In some cases, whisker tags fell off and were thus not always present. Whisker movements were analyzed using nose, whisker base and tag positions.

2.9 Data analysis

2.9.1 Correlation of the V_m and whisking (chapter 3)

To detect locking of the V_m to the subject rat's whisking, protraction triggered V_m averages were calculated in Matlab by averaging the V_m ± 100 ms relative to the minima of the whisker motion trace. V_m values were averaged over an entire interaction episode.

Modulation depth values were calculated by subtracting the minimum from the maximum of the protraction triggered V_m curves. The mean modulation depth was computed as an average of modulation depth values of individual episodes. Whisking amplitudes and set angles were automatically calculated using the custom-written Matlab code for whisker tracking. Correlation coefficients were computed in Matlab using the Spearman rank correlation test. All other statistical tests (Mann-Whitney U and Wilcoxon signed-rank test, Kruskal-Wallis One-Way ANOVA) were performed in Matlab. Mean V_m and firing rates were calculated over the total length of a given episode.

2.9.2 Quantification of somatosensory areas and sizes (chapter 4)

The area of somatosensory regions was measured by drawing an outline around the anatomical region of interest and calculating its area using the ImageJ area calculating tool. The area of the following cortical representations was measured: hind-paw, forearm, trunk, penis, and clitoris. In addition, various aspects of the size of the cortical genital region were determined by measuring the length from the tip of the genital representation to its base (shaft length), the width half way from the base (half-width), and the length from the tip to the back of the trunk (total genital length) (see Fig.4.4). In order to get complete body maps (as shown in Fig.4.8) body outlines and barrels were drawn through multiple serial tangential cortical sections using a computer-aided system (Neurolucida, MBF Bioscience, Vermont, USA).

2.9.3 Matching anatomical maps to physiological response maps (chapter 4)

For the purpose of matching anatomical maps to the physiological maps three different methods were used. All of them led to the same conclusions. First, electrolytic lesions (n = 2) were performed in order to match the individual recording sites to the anatomical map locations. This was done by injecting 10 μA negative current through a tungsten electrode for 10 s. Second, individual physiological maps were matched to the overall layout of individual anatomical maps (n = 10). Third, anatomical maps (n = 17 hemispheres/maps from young animals and n = 9 hemispheres/maps from adult animals) were matched to our overall maps (Fig.4.2E and F) and published maps (Chapin & Lin 1984; Welker 1971).

2.9.4 Analysis air-puff stimulation during juxtacellular and whole-cell patch clamp recordings in the genital cortex (chapter 4)

In order to analyse air-puff stimulation responses obtained during juxtacellular recordings in the genital cortex, traces were exported to Spike2 and a DC filter was applied. This allowed the optimal monitoring of evoked spikes. Spike time points were calculated by setting different detection thresholds in Spike2 and exported afterwards to Matlab. Using an adapted code, response trials were aligned to the stimulus onset and Peri-Stimulus-Time-Histograms (PSTHs) were plotted. For pooled PSTHs only cells with ongoing firing rates greater than 0.2 Hz were included and PSTHs were normalized such that each cell contributed equally (normalized PSTHs).

For the analysis of the whole-cell recordings HEKA files were exported directly to Matlab and trials were aligned to the stimulus onset and averaged.

Chapter 3

Barrel Cortex Membrane Potential Dynamics in Social Touch

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Abstract

Facial interactions are important for many species, including rats, which use them to communicate food preferences or other behavioral states as aggression. The whisker-to-barrel system is an attractive model to study the neuronal processing of active touch. In most of these studies, artificial stimulation of the whiskers is used, even though responses to facial touch appear to be quite different when more natural, socially evoked stimuli are given by the context. While the correlation of V_m and rhythmic whisking has been analyzed in the somatosensory cortex during free whisking, we know little about the impact of active touch and the consequences of meaningful stimuli on the V_m dynamics of barrel cortex neurons. To address this question we obtained *in vivo* whole-cell recordings in the barrel cortex of head-restrained rats. We studied barrel cortex responses during social interactions, in which the experimenter presented a stimulus rat to the head-fixed subject rat. Whisking behavior was quantified by tracking whisker angles in the subject rat using videography. Social touch was associated with a depolarization and large V_m fluctuations locked to the rat's whisking. Both depolarization and V_m fluctuations were already observed prior to contact and did not occur during free whisking. This anticipatory pre-contact depolarization was not seen in passive social touch in anesthetized animals. The V_m fluctuations locked to the rat's whisking observed in interactions with awake conspecifics were larger than those seen for whisking onto non-conspecific stimuli (stuffed rats, objects, and the experimenter's hand). Responses did not correlate with whisker movement parameters. Taken together we conclude that responses to social touch differ from conventional tactile responses in amplitude, locking to whisking, and pre-contact V_m changes.

Chapter 4

Sexually Monomorphic Maps and Dimorphic Responses in Rat Genital Cortex

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Abstract

Genitals are sexually dimorphic body parts with poorly understood neural representations. We identified genital responses in rat somatosensory cortex in a region previously assigned as arm/leg cortex. Genital responses were more frequent in males than in females. Despite such response dimorphism, we observed a stunning anatomical monomorphism of cortical penis and clitoris input maps detected by cytochrome-oxidase staining of cortical layer 4. Cortical genital maps were bilaterally symmetric and larger than arm/leg in area but smaller than paw representations. Interestingly their relative size increased markedly during puberty. Size, shape and erect posture give the cortical penis representation a phallic appearance pointing to a role in sexually aroused states. Cortical genital neurons had unusual, discontinuous and sexually dimorphic receptive fields. Specifically, genital neurons were co-activated by distant body regions which are touched during mounting in the respective sex. Genital maps indicate a deep cortical homology of penis and clitoris representations in line with a fundamentally bi-sexual layout of the vertebrate brain.

Chapter 5

General Discussion

We know a great deal about the processing of simple sensory stimuli in the barrel cortex (Petersen 2007). Similarly, we have relatively detailed insights into the brain structures involved in spatial navigation. At the same time we know little about the forebrain structures that mediate social interactions and at least when it comes to cellular underpinnings the field of social neuroscience is at an early stage. So far most of our knowledge about sensory processing stems from precisely controlled and well planned experiments, where the subject (human or animal) is placed in a reduced artificial setting. By contrast, in the natural environment, humans and a great proportion of animals spend a lot of time interacting with other individuals. Nevertheless, setups are used in social neuroscience, where different pictures qualified as non-social or social are presented to the subject while recording brain activity. Since most studies using human subjects apply functional imaging in an fMRI scanner, it is of course difficult to set up a naturalistic social setting. Meanwhile we can avoid such methodological difficulties using animal models to examine the basic neural mechanisms underlying normal social behavior on the one hand and also investigate abnormal behavior and brain function in animal models of mental or neurodegenerative disorders on the other hand.

Rats are highly social animals and thus an excellent model system for the field of social neuroscience. They express many biologically relevant behaviors, including sexual behavior and maternal care. For many of these behaviors touch is an important sensory cue. Prior to this thesis we had only little information about the activity of primary somatosensory cortex in social contexts. Social interactions involve the transmission of many sensory cues, such as olfaction, vision or audition. Therefore it seems likely that tactile stimuli carry meaningful information in a social context and thus may be processed differentially in the primary somatosensory cortex.

In my thesis I studied how primary somatosensory cortex is modulated by two relevant forms of touch. The first part aimed at investigating the modulation of the V_m of barrel cortex neurons during social facial touch. The second part combined physiological and anatomical methods in order to investigate a somatosensory representation of the rat

genitals. Further, the study aimed at characterizing how simple genital touch is processed in the genital cortex of male and female rats.

5.1 Barrel Cortex Membrane Potential Dynamics in Social Touch

In the first part of this thesis, an experimental design was applied, where an actual social interaction between individual animals could be monitored in parallel with the recording of brain activity. Specifically, the activity of single cells in somatosensory cortex was recorded while animals were allowed to facially touch each other. The modulation of S1 activity during social facial touch was closely examined and compared to that during active touch of objects. Interestingly, our findings suggest that S1 activity does not simply reflect the mechanical patterns of tactile stimuli (Lenschow & Brecht 2015). The V_m modulations and locking to the subject's whisking were substantially bigger during interactions with conspecifics compared to interactions with non-social stimuli. Yet, how these differences evolve remains unclear. The multisensory cues (Galef 2013) might contribute to the unique responses of barrel cortex neurons to social touch. Direct connectivity has been shown between somatosensory and auditory cortices (Budinger et al. 2006) suggesting that ultrasonic calls could shape barrel cortex activity. Another study investigated the modulation of somatosensory cortex of lactating anaesthetized female mice by pup odors and found no changes in activity (Cohen et al. 2011) arguing against olfactory effects on activity of the S1. If such results hold up during wakefulness remains to be determined.

As barrel cortex responses are shaped by the state of alertness and arousal (Bosmann et al. 2011), the possibility that social stimuli excite the subject rat more than objects cannot be ruled out. It needs to be mentioned, however, that animals were trained to the experimental condition and were aware of the possible appearance of either objects and/or conspecifics. Further it is worthwhile investigating whether responses more downstream in the information processing (i.e. in the VPm of the thalamus) are already modulated by social tactile stimuli and whether this modulation differs from responses induced by object touch. The same applies to the effect seen prior to a social interaction: V_m depolarization is observed before the animals are touching. Moreover, there are studies pointing to barrel cortex activation by sniffing (Ito et al. 2014). Hence, we predestine socially induced sniffing as a potential factor accounting for the

pre-contact depolarization seen in our results. Whereas the study by Bobrov et al. (2014) reported sexual differences in the barrel cortex of rats, which were in addition modulated by the sexual state of the subject rat, we could not make out a clear sex-specific modulation of V_m dynamics. Since the animals used in our study were aged between P30 and P35 and thus considerably younger and sexually immature than in the study by Bobrov et al. (2014), it is difficult to directly compare the results of these two studies. For getting a better insight if and how V_m dynamics are shaped by the sex of the subject/and or stimulus rat plus the sexual state of subject females, whole-cell patch clamp recordings should be done in adult animals. Recordings in adult animals, however, are harder to achieve. Also patching over a full estrus cycle would of course give an excellent resolution, but is quiet challenging, too.

The findings on social facial touch are of interest for four reasons. First, our study used whole-cell patch clamp recordings in awake animals during a social setting. Thus, an excellent resolution of subthreshold events during social whisking is obtained. Second, it showed a differential modulation on the cellular basis of S1 during social facial touch compared to free whisking. Third, the activation of barrel cortex neurons before an actual tactile input, is an unexpected finding, which calls for further investigation. Fourth, the stronger modulation of barrel cortex V_m during social touch compared to non-conspecific touch, confirms a differential processing of S1 during biological meaningful behaviors. Taken together, the results suggest that social sensory stimuli are processed differentially from non-social stimuli already in primary cortical areas.

5.2 Sexually Monomorphic Maps and Dimorphic Responses in Rat Genital Cortex

Touch in general carries important information during many behavior patterns and facial touch turned out to be especially pronounced in rodents. Genital touch is at least as important and even more crucial during reproductive behavior. It has been described mostly in the context of sexual behavior and the underlying circuits of those are studied extensively in rats and mice (Bergan et al. 2014, Lee et al. 2014, Nomoto and Lima 2015). The impact of sensory cues transmitted by the genitals, however, is somehow ignored in those studies. Given the remarkable sexual dimorphism of external genitals, it is of particular interest whether the dimorphism holds in their sensory representation. Even though, multiple studies have aimed at mapping the

sensory receptivity of the genitals onto the human cortex (Penfield and Rasmussen 1950, Rothemund et al. 2002, Komisaruk et al. 2011), there are still controversies about the exact position (Di Noto 2013). Animal studies also lack a distinct sensory or motor representation of the genitals. Only an old mapping study in rabbits, included genitals in the body map, although at an unusual position (Gould 1986).

Hence, the second study of this thesis aimed at identifying the somatosensory representation of the genitals in the rat cortex and further at investigating the processing of simple stimuli within the genital cortex of male and female rats. Using the old fashioned cytochrome oxidase staining in flattened cortical preparations after having mapped genital responses in the somatosensory cortex of anaesthetized animals, a clear delineation of genital somatosensory cortex was revealed, in both males and females. To our surprise, the genital somatosensory cortex appeared to be sexually monomorphic even though external genitals show remarkable dimorphism. In addition to extending the 'ratunculus' by including the missing genitals, we also reported differential sexual responses to simple air puff stimuli in the genital cortex of females and males. In line with Bobrov et al. (2014), who reported a weaker modulation of barrel cortex neurons recorded in females by social facial touch, we found weaker responses in the female genital cortex to palpitation. How these responses come about remains elusive, but there is a strong suggestion that the activity of somatosensory cortex is hormone dependent (Bobrov et al. 2014). Since prepubescent animals were used for recordings in our project, conducting mapping experiment and/or single-unit recordings while applying air-puffs in different estrus phases of adult female rats is advisable. The second surprising and sexually dimorphic finding was the composition of receptive fields with different body parts. While neuronal responses recorded in genital cortex were co-activated by the sensory stimulation of the trunk, multi-units recorded in male genital cortex, were not only activated by genital stimulation but also by forearm sensory stimuli. This gives rise to the idea that such sexually dimorphic receptive fields might reflect a sexual function: the body parts co-represented with genitalia are those parts contacted in males and females during mounting (Barnett 1963). These findings call for electrophysiological recordings in awake behaving animals which can shed light into the processing of genital touch in male and female rat's somatosensory cortex.

Since anatomical and mapping experiments were performed in prepubescent animals, we wanted to confirm the clear delineation of our body maps, in adult animals by

conducting cytochrome oxidase stains and quantifying the areas of the rat homunculi. Thereby we observed a substantial growth of the genital cortex during puberty, which is quite unusual given the immutable and rigid layout of layer 4 input maps after birth (Van der Loos and Woolsey 1973, Feldman and Brecht 2005). Whether this growth underlies hormonal control needs further attention. Taken together, the results obtained are of great significance for sexual behavior research in animals and encourage further anatomical and functional investigation of the genital cortex, especially in the context of sexual information flow in the brain.

5.3 Overall conclusion

This thesis investigated the role of primary somatosensory cortex in the cellular processing of social facial touch (Chapter 3) and identified rat genital somatosensory cortex (Chapter 4), which showed sexual dimorphic responses. The first study showed that high-level tactile and non-tactile social information is represented in the barrel cortex and therefore challenges the view that somatosensory cortex is primarily concerned with the mechanical properties of stimuli. The second study identified for the first time the position of the rat genital somatosensory cortex. Surprisingly rat genital cortex turned out to be anatomically sexually monomorphic. It was also found that the genital somatosensory cortex is functionally different in males and females. In their own ways, both studies provide motivation for further research of facial and sexual touch in the somatosensory cortex and beyond.

Abbreviations

A	anterior
fMRI	functional Magnetic Resonance Imaging
IR	infrared
L	lateral
LH	Left hemisphere
M	medial
ms	millisecond
mV	millivolt
PFA	Paraformaldehyd
Pom	posterior medial nucleus of the thalamus
P	posterior
P(20)	postnatal day 20
PSTH	Peri-Stimulus-Time Historgram
RH	Right hemisphere
S1	Primary somatosensory cortex
SD	standard deviation
SEM	standard error of the mean
t-Test	student's t-distribution test
V_m	Membrane potential
VPm	ventral posterior medial nucleus of the thalamus

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Statement of Contribution

Since the results presented in Chapter 4 of my doctoral thesis were obtained in a collaborative approach, a short statement of the relative contributions to the data is given. The project was initially started during the Neural Systems and Behavior summer course at the Marine Biological Institute in Woods Hole. The students of the course Sean Copley, Jayne M. Gardiner, Zoe N. Talbot and Ariel Vitenzon performed the first experiments of the study together with my PhD supervisor Prof. Michael Brecht and me. In the following I performed further experiments concerning the anatomy of adult genital maps and properties of receptor fields. I also performed the data analysis of these experiments. Both projects presented in this thesis resulted in Publications, which are listed below. In this context it should be mentioned that the whole publication process (including revision experiments) was conducted by my PhD supervisor and me.

Publications

Lenschow, C., Brecht, M. (2015). Barrel cortex membrane potential dynamics in social touch. Neuron *85*, 718-725.

Lenschow, C., Copley, S., Gardiner, J., M., Talbot, Z., N., Vitenzon, A., Brecht, M. (2016). Sexually Monomorphic Maps and Dimorphic Responses in Rat Genital Cortex. Curr Biol. *26*, 106-113.

Eigenständigkeitserklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Ich erkläre, dass ich sämtliche in der oben genannten Arbeit verwendeten fremden Quellen als solche kenntlich gemacht habe und gemäß den mir bekannten gängigen wissenschaftlichen Regeln korrekt zitiert habe. Ich bestätige, dass ich bei wörtlich übernommenen Aussagen bzw. bei unverändert übernommenen Grafiken als auch bei in eigenen Worten wiedergegebenen Aussagen bzw. von mir abgewandelten Grafiken anderer Autoren die Quelle angegeben habe. In dem Zusammengang, muss erwähnt werden, dass die in der vorgelegten Arbeit behandelten Themen bereits in wissenschaftlichen Zeitungen veröffentlicht wurden. Aus diesem Grund sind Ähnlichkeiten in Text und Abbildung unvermeidbar. Zu Beginn eines jeden Kapitels, wurde deshalb auch noch einmal explizit darauf verwiesen, dass das Thema bereits veröffentlicht wurde.
Ich habe diese Arbeit nicht anderweitig zu Prüfungszwecken vorgelegt und habe mich nicht anderwärts um einen Doktorgrad beworben. Des weiteren versichere ich, dass keine Zusammenarbeit mit gewerblichen Promotionsberatern statt gefunden hat. Bei Anfertigung der Arbeit wurden die Grundsätze der Humboldt-Universität zu Berlin zur Sicherung guter wissenschaftlicher Praxis eingehalten. Die dem Promotionsverfahren zugrunde liegende Promotionsordnung ist mir bekannt.

Datum:

Unterschrift: